larger as the chemical transmission is more active. The findings up to 12 h after CP administration support some previous studies. Various neuroleptics accelerate loss of fluorescence of dopaminergic terminals in the caudate nucleus when tyrosine hydroxylase is inhibited[8]. Increase of synaptic vesicles in 24 h after CP administration suggests a possibly inhibited release of transmitter or accelerated reuptake or synthesis of monoamine for its usual consumption. Because CP prevents uptake of monoamine to synaptosome[9], and inhibits the electrically stimulated release of H^3-dopamine from rat striatal slices[10], the present findings in 24 h after CP administration suggest that CP will lead to a condition in which neural transmission is inactive. In other words, CP is thought to cause clinical effects through the presynaptic blocking in part of its actions.

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Are intermediate filaments of vertebrate smooth muscle cells and tonofilaments of epithelial cells identical cell structures?

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Summary. In epithelial and smooth muscle cells of the urinary bladder of the frog, a class of filaments exists which is partly disintegrated by glycerol treatment and very resistant to potassium solutions of high ionic strength.

There are some indications that the intermediate filaments found in different muscle types and the tonofilaments described in a number of non-muscle cells are similar cell structures. Both have a diameter of about 100 Å[2-8], are resistant to various extraction procedures[5,9] and cannot be decorated with heavy meromyosin[4]. Since, however, investigations were made either on muscle or on non-muscle cells using different species and procedures, the results are not really comparable. In the present report, a tissue is used which contains muscle and epithelial 100 Å filaments in immediate vicinity. Therefore, fixation and extraction procedures were carried through under identical conditions.

Material and methods. The tissue employed was the urinary bladder of the frog, *Rana temporaria*. Small strips of the bladder were first incubated for 20 h in ice-cold 50% glycerol, 10^{-2} M $MgCl_2$, 10^{-2} M Tris-HCl (pH 7.2) solution. For salt extraction, glycerinated tissue was placed in one of the following extraction solutions for 5 h[10]: A 4 mM EGTA, 4 mM $MgCl_2$, 5 mM ATP, 0.05 M KCl, histidine

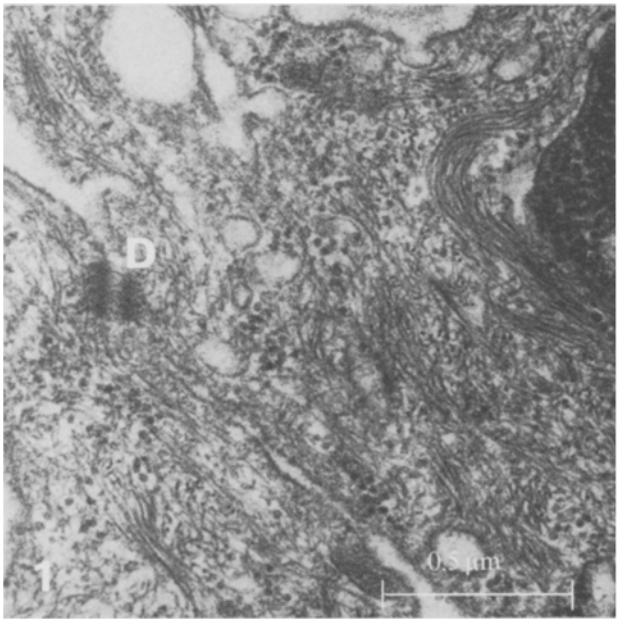

Fig. 1. Epithelial cells of the urinary bladder of the frog. The cells contain numerous tonofilaments, running through the cytoplasm at all angles. D = desmosome.

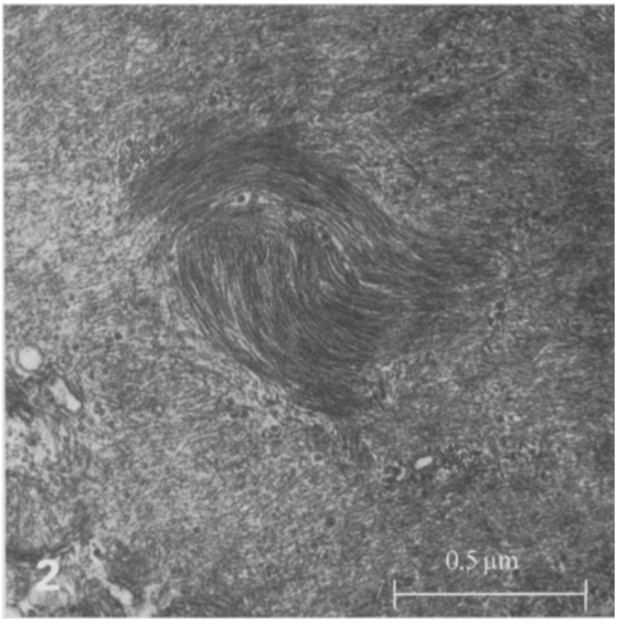

Fig. 2. Oblique section through a smooth muscle cell of a bladder. In the centre of the cell a large bundle of darkly-stained intermediate filaments is seen.

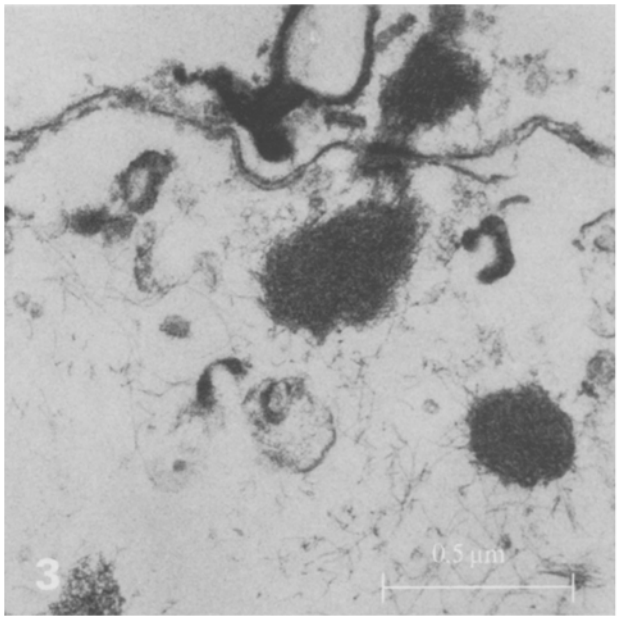

Fig. 3. Epithelial cell, treated with glycerol and extraction solution B. Clumps of tonofilaments are still present.

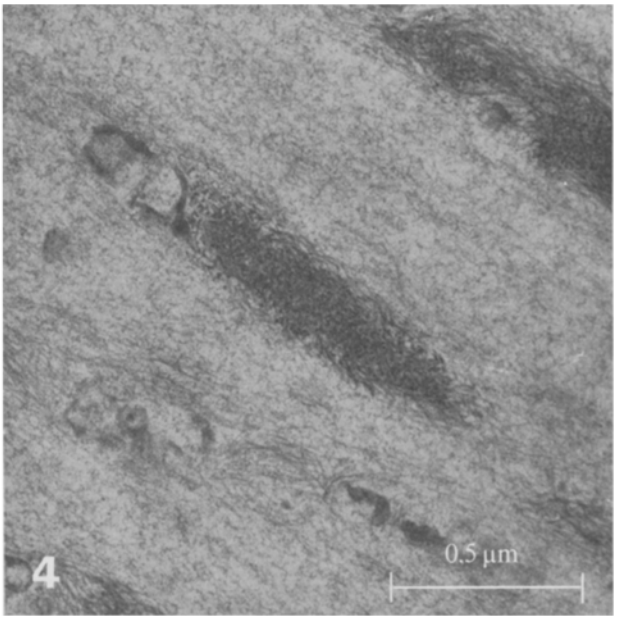

Fig. 4. Muscle cell, same treatment as figure 3. Only clumps of intermediate filaments are seen.

buffer pH 7.2. B 4 mM EGTA, 4 mM $MgCl_2$, 5 mM ATP, 1 M KCl, histidine buffer pH 7.2. C 0.15 M KCl, histidine buffer pH 7.2. Glycerinated or salt extracted strips were fixed with 5% glutaraldehyde and postfixed with 1% osmium tetroxide. For structural studies fresh urinary bladders were fixed in the same manner. The tissue was embedded in an Araldite-Epon mixture, stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 101 electron microscope.

Results and discussion. As figure 1 shows, the tonofilaments (diameter 100 Å) of epithelial cells are mostly arranged in bundles which run in random directions throughout the cytoplasm. Sometimes bundles disaggregate to a network of single filaments. The lateral plasma membrane exhibits a desmosome in which tonofilaments were seen to converge. Intermediate filaments (diameter 90–100 Å) occur in all vertebrate smooth muscles so far examined in a relatively small number among the actin filaments. Contrary to these observations, they form in the muscle cells of the urinary bladder of the frog compact bundles of up to several hundred filaments (figure 2). Besides these filament concentrations smaller bundles and meshworks of intermediate filaments were distributed between the actin filaments.

In order to decrease the diffusion barriers for the salt solutions urinary bladders were first extracted with glycerol. This treatment caused characteristic disruptions of cell structures like plasma membrane, mitochondria or endoplasmic reticulum. More interesting is the fact that glycerol treatment changes the appearance of the 100 Å filaments in both cell types in the same way. In regions normally occupied by bundles of the 100 Å filaments, clumps of granular to filamentous material occur. It seems that these clumps represent the population of the 100 Å filaments which were partly disintegrated and clustered together during glycerination. The structural aspect of the actin filaments was not changed by glycerol treatment. When the glycerinated bladders were treated with salt solutions, different effects could be observed. Incubation in a 0.15 M KCl solution did not affect actin filaments nor 100 Å filaments, compared with samples which were only glycerinated. However, extraction with solution A or B removed the actin filaments, whereas the clumps of the 100 Å filaments survived these procedures (figures 3, 4).

The data presented in this report indicate that a class of filaments occurs in muscle cells and epithelial cells of frog urinary bladder which exhibits a number of corresponding features. They are characterized by a diameter of 90–100 Å, an arrangement in bundles and a similar behaviour in various chemical treatments. Glycerination of tissue often results in a loss of individual filaments which cluster together to granular or filamentous condensations. Furthermore, the 100 Å filaments are very resistant to KCl solutions of high ionic strength, procedures which extract filaments composed of actin or myosin. Additional biochemical data, however, are required to permit the conclusion that intermediate filaments of smooth muscle cells and tonofilaments of epithelial cells are identical structures.

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